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Salinity and osmotic stress trigger different antioxidant responses related to cytosolic ascorbate peroxidase knockdown in rice roots

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ABSTRACT

Salinity and osmotic stress trigger distinct signals in roots, which might induce differences in antioxidant responses. To clarify these relationships, transgenic rice plants silenced in both cytosolic ascorbate peroxidases (*apx1/2*) and non-transformed (NT) were exposed to iso-osmotic concentrations of NaCl and mannitol. Under both stress conditions, *apx1/2* roots did not suffer oxidative stress, revealing that cytosolic APXs were not crucial to oxidative protection. Silenced and non-transformed roots triggered different responses to high salinity and osmotic stress and these stressful factors induced also distinct antioxidant changes. High salinity up-regulated expression of important *OsAPX* isoforms and these changes were related to increased APX activity, especially in NT roots. Intriguingly, salt stress triggered up-regulation of *OsCAT* isoforms but CAT activity did not change in both genotypes. In contrast, mannitol trigged very low increment in expression of *OsAPX* isoforms but induced substantial up-regulation in APX activity in NT roots. Mannitol also remarkably up-regulated *OsCATB* expression in parallel to CAT activity, in both *apx1/2* and NT roots. POD and GPX (glutathione peroxidases) activities were strongly increased by high salinity but did not change in response to mannitol, in both genotypes. The two stress types as well as *apx1/2* and NT roots displayed different response in terms of modulation in the H_2O_2 levels but lipid peroxidation did not change. Membrane integrity was drastically affected by both stressful factors and similarly in both genotypes, whereas root fresh matter was affected only by salt stress. Altogether, the obtained data reveal that high salinity and osmotic stress trigger different antioxidant responses and these strategies were genotype-dependent. The different antioxidant molecular-biochemical mechanisms employed by cytosolic APX knockdown and non-transformed roots allowed reaching similar physiological performance.

1. Introduction

Plant roots play critical roles during growth and development, such as mineral nutrient uptake, maintenance of adequate plant water balance and hormone synthesis (Pierik and Testerink, 2014). Moreover, roots are intrinsically related to stress perception and signaling mechanisms connecting soil factors with whole plant metabolism (Choi et al., 2014). Soils are important sites of specific abiotic stresses, such as water deficit, high salinity, toxicity, nutrient deficiency, flooding etc. (Gupta et al., 1999; Małecka et al., 2001; Zhang and Zhang, 1994; Tabata et al., 2014). Most of these stressing factors have as a common characteristic the generation of a secondary stress related to disturbances in the redox metabolism (Adem et al., 2014). This secondary oxidative stress in roots is resultant from an unbalance between production and scavenging of reactive oxygen species (ROS) (Adem et al., 2014; Cavalcanti et al., 2007).

The mechanisms involved with generation and elimination of ROS in roots under high salinity and osmotic stress are scarcely known (Adem et al., 2014; Cavalcanti et al., 2007; Hernandez et al., 1993). High salinity has two components; ionic toxicity and osmotic and these elements might induce different types of responses in roots (Flowers et al., 2015; Munns and Gilliham, 2015;

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Roy et al., 2014). Both saline and osmotic agents might generate disturbances in root metabolism, including deleterious effects in several organelles and cellular compartments (Flowers et al., 2015; Zhu, 2002). These alterations might affect especially the cell wall, apoplast, cytosol and mitochondria, inducing changes in gene expression and enzyme activities related to redox metabolism (Maia et al., 2013). Under these conditions, frequently the production of ROS is over increased and the antioxidant mechanisms might be insufficient to re-establish a favorable redox homeostasis (Foyer and Noctor, 2015).

The molecular-biochemical mechanisms involved with root perception of signals from saline ions and osmotic stress are complex and still poorly understood (Choi et al., 2014). After perception and signal transduction in cytosol, a cascade of reactions occurs until the expression of different stress-related genes (Maathuis, 2014). These genes encode for several important proteins including those associated with root growth and ROS scavenging (Mittova et al., 2004). ROS production in roots might occur from biochemical and chemical processes such as Haber-Weiss-Fenton reactions (Halliwell and Foyer, 1976), excess energy in mitochondrial electron transport chain – ETC (Møller, 2001), up-regulation of NADPH oxidase in plasmalemma (Passardi et al., 2004) and alterations in the cytosolic ascorbate-glutathione cycle (Munné-bosch et al., 2013).

Salt and osmotic stresses might induce down- or up-regulation in the expression of genes that encode for catalases (CAT), ascorbate peroxidases (APX) and glutathione peroxidases (GPX) and type III peroxidases (POD) (Hong et al., 2009, 2007; Mittova et al., 2004). APX and CAT are the most important peroxidases responsible for scavenging and maintaining of H_2O_2 in levels adequate to redox homeostasis in roots (Cavalcanti et al., 2007). Rice has eight APX isoforms, viz. two localized in mitochondria, two in cytosol, two in chloroplast and two in peroxisomes (Teixeira et al., 2006, 2004). The cytosolic APX isoforms in plants are found in high concentrations and these enzymes are strongly involved in protection against abiotic stress in leaves (Shigeoka and Maruta, 2014). These enzymes are the most important members of APX family in antioxidant protection (Shigeoka and Maruta, 2014).

The role and importance of cytosolic APX isoforms as well as its relationships with other peroxidases in root redox metabolism is incipient (Maia et al., 2013). The antioxidant metabolism in roots, especially in response to ionic toxicity and osmotic stress, is much less studied compared to leaves. The majority of published articles concerning the saline and osmotic stress responses in roots are descriptive and the underlying mechanisms involving the role of each specific antioxidant are poorly understood (Cavalcanti et al., 2007; de Azevedo Neto et al., 2006; Maia et al., 2013). For instance, which is the importance of cytosolic APXs and CAT in scavenging and maintaining of H_2O_2 homeostasis in roots exposed to acute salinity and osmotic stress? In addition, which is the role, complementary or primary, of other peroxidases such as CAT, POD and GPX in such processes? Undoubtedly, the knowledge on redox metabolism generated from leaf studies would not be extensible for roots since that these organs present very different structures related to oxidative and antioxidant metabolism.

We hypothesized here that high salinity and osmotic stress trigger different antioxidant responses in rice roots and these differences also are distinct among cytosolic APXs deficient (knockdown) and non-transformed rice roots. This study revealed that ionic and osmotic stress triggered very different antioxidant responses represented by the differential modulation in *OsAPX* and *OsCAT* expression and regulation of APX, CAT, GPX and POD activities. The role of H_2O_2 in these responses and the physiological significance in terms of oxidative in roots is discussed.

2. Materials and methods

2.1. Construction of the plant vector and plant transformation

The non-transformed (NT) and transgenic (*apx1/2*) rice (*Oryza sativa* L. cv. Nipponbare) plants were obtained as previously reported by Rosa et al. (2010). Chimerical gene producing mRNA with a hairpin structure (hpRNA) was constructed based on the sequence of the *OsAPX1* (LOC_Os03g17690) and *OsAPX2* (LOC_Os07g49400) genes. The following primer pairs were used: CGCCGCCAACGCCGGCCTCGA and CACTCAAACCCATCTGCGCA (*OsAPX1/2*RNAi). PCR products were cloned into the Gateway vector (pANDA), in which hairpin RNA is driven by a maize ubiquitin promoter and an intron that is placed 50 bp upstream of inverted repeats (Miki and Shimamoto, 2004). Agrobacterium mediated transformation was performed as described previously (Rosa et al., 2010). After an initial screening involving 15 lines, three were selected (*apx1/2*-5, *apx1/2*-10 and *apx1/2*-11). These lines showed similar molecular and physiological characteristics (Rosa et al., 2010). Previously (Bonifacio et al., 2011), and in this study, the *apx1/2*-5 line was used, at the F3 generation, as a representative mutant of the *apx1/2* double-silenced lines. These plants have exhibited a similar response in terms of *OsAPX1/21* and *OsAPX2* transcript amount and APX activity in leaves at F1, F2 and F3 generations (Bonifacio et al., 2011).

2.2. Plant growth and treatments

apx1/2 and the NT seedlings (7-day-old) were transferred to 3 L plastic pots filled with half-strength Hoagland-Arnon's nutritive solution (Hoagland and Arnon, 1950). The pH was adjusted to 6.0 ± 0.5 every two days, and the nutrient solution was changed weekly. The plants were grown for 45 days in a greenhouse under natural conditions as follow: day/night mean temperature of 29/24 °C, mean relative humidity of 68%, and a photoperiod of 12 h. The light intensity inside the greenhouse varied as a typical day from 6:00 a.m. to 6:00 p.m., reaching an average of maximum PPFD equals to 820 $\mu mol\ m^{-2}\ s^{-1}$ at noon. NT and *apx1/2* plants were grown in nutrient solution supplied with NaCl and mannitol, which were used in iso-osmotic concentrations corresponding to −0.62 MPa. The osmolality was measured in a vapour pressure osmometer (Model 5520, Wescor®, USA) and the final concentrations of NaCl and mannitol were adjusted to 150 mM and 268 mM, respectively. The nutrient solution without these two solutes was used as control. NaCl and mannitol were added to the nutrient solution in two steps (half of each solute per day) to avoid osmotic shock. Plants were subjected to these stressful conditions for eight days. Subsequently, 5 cm from the superior part of mature rice roots were immediately harvested, frozen in liquid N_2 and stored at −80 °C until the biochemical and transcript analyses.

2.3. Na^+ and K^+ content determinations

The Na^+ and K^+ contents in roots were determined as previously described (Marques et al., 2013). Lyophilized root samples were transferred to hermetically sealed tubes containing deionized water. Subsequently, the samples were boiled in water bath at 100 °C for 1 h. After extract filtration using filter paper, the Na^+ and K^+ contents were determined by flame photometry (B462, Micronal®, Brazil).

2.4. H_2O_2 concentration, membrane damage (electrolyte leakage) and lipid peroxidation (TBARS content)

Hydrogen peroxide content was measured using the Amplex®-red kit (Thermo Fisher Scientific®, USA), based on colorimetric